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Polyinosinic-Polycytidylic Acid Complexed with Poly-L-lysine and Carboxymethylcellulose in Combination with Interleukin 2 in Patients with Cancer: Clinical and Immunological Effects[1]

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ABSTRACT

We have performed a phase IB study of polyinosinic-polycytidylic acid complexed with poly-L-lysine and carboxymethylcellulose (poly-ICLC) in combination with interleukin 2 (IL-2) in 25 patients with a variety of cancers. Patients received weekly or biweekly poly-ICLC by i.m. injection, at doses ranging from 0.01 to 1.0 mg/m^2, for 1 month. This was followed by 2 months of outpatient therapy with biweekly i.m. poly-ICLC in combination with IL-2 (3×10^6 units/m^2) given i.v. by 24-h continuous infusion twice weekly, using a portable infusion pump. No objective tumor responses were observed. Toxicity was moderate at all poly-ICLC doses tested and increased only slightly following the addition of IL-2. No increases in peripheral blood natural killer (NK) activity were observed after treatment with poly-ICLC alone. However, high dose poly-ICLC ($\geq$0.3 mg/m^2) in combination with IL-2 resulted in NK activity greater than that seen using the same dose of IL-2 in combination with lower poly-ICLC doses. Increases in the number and percentage of CD56$^+$ cells were evident only after initiation of IL-2 therapy and were unaffected by the poly-ICLC dose. In the majority of patients, these increases were preferentially associated with the subset of CD56$^+$ cells coexpressing CD8, while the CD56$^+$/CD16$^+$ population was elevated to a lesser extent. Moderate increases in serum neopterin levels and 2′,5′-oligoadenylate synthetase activity in peripheral blood mononuclear cells were noted at 72 h following initial treatment with 1.0 mg/m^2 poly-ICLC. No induction of α or γ interferon was detected. This study shows that the addition of poly-ICLC to a well tolerated IL-2 regimen can significantly enhance NK activity. Poly-ICLC can be used to enhance IL-2-induced NK lytic activity without increases in the dose and, therefore, the toxicity of IL-2 treatment.

INTRODUCTION

Poly-ICLC[3] is one of a family of synthetic double-stranded RNA molecules known to be potent interferon inducers. Animal studies have shown that this prototypic biological response modifier has a variety of immunological and antitumor effects (1, 2). Indeed, poly-ICLC is such a potent immune enhancer that it is routinely used as a positive control in preclinical studies testing other biological agents. Despite demonstrable antitumor activity in animal models, poly-ICLC has not produced significant antitumor effects in humans. One reason for this lack of efficacy may be that no optimal dose, route, or schedule of poly-ICLC administration for immunomodulatory effects has been established. This may be important, because poly-ICLC is postulated to mediate its antitumor effects via immune rather than direct cytotoxic effects. Previous clinical trials (3) have concentrated on determining a MTD rather than an optimal immunomodulatory dose. Experimental animal data suggest that maximal antitumor effects coincide with optimal NK cell and macrophage activation by poly-ICLC, which are achieved at doses considerably below the MTD (4).

The MTD for poly-ICLC in humans is approximately 12 mg/m^2, but lower doses of poly-ICLC produce immunomodulatory effects in cancer patients (5). Although no antitumor responses were observed, measurable changes in monocyte function and the IFN-inducible enzyme 2–5A, as well as elevation of NK activity, were observed in patients receiving 1 mg/m^2 poly-ICLC. In addition, patients treated at 1 mg/m^2 were more likely to have increased NK activity than were patients treated at higher doses, where decreased NK activity was frequently observed. Thus, the optimal immunotherapeutic and immunomodulatory dose in humans may be $\leq$1 mg/m^2.

Interleukin 2 can increase the number and activity of NK cells (6), LAK cells (7), cytotoxic T lymphocytes (8), and tumor-infiltrating lymphocytes (9). It has antitumor effects when administered alone (10, 11) or in combination with LAK cells (12) or tumor-infiltrating lymphocytes (13). In a study of Rosenberg *et al.* (12), six of 46 (13%) cancer patients receiving high dose IL-2 (100,000 units/kg, three times per day) achieved a complete or partial response. Treatment was accompanied by severe toxicities requiring hospitalization, often in an intensive care setting. An outpatient regimen employing daily infusions of low dose IL-2 (3×10^6 units/m^2, by 24-h continuous infusion) has been found to increase the number of circulating LAK cells, with tolerable toxicity (14). We wondered if the antitumor activity of IL-2 might be enhanced if it were given in combination with a second biological response modifier, such as poly-ICLC, which has the potential to enhance similar immune functions. This study was designed to investigate the toxicity and the immunomodulatory effects of low dose poly-ICLC alone and to explore the possibility of synergistic or additive immunological activity of poly-ICLC combined with IL-2.

PATIENTS AND METHODS

Patients. Twenty-five patients with a variety of malignancies (Table 1) were entered into the study. All patients were required to meet the

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[3] The abbreviations used are: poly-ICLC, polyinosinic-polycytidylic acid complexed with poly-L-lysine and carboxymethylcellulose; NK, natural killer; LAK, lymphokine-activated killer; IL-2, interleukin 2; IL-2R, interleukin 2 receptor; PBS, phosphate-buffered saline; PBM, peripheral blood mononuclear cells; MTD, maximal tolerated dose; 2–5A, 2′,5′-oligoadenylate synthetase.

Table 1 *Patient characteristics*

No. of patients entered	25
Male:female	13:12
Median age in years (range)	54 (26–69)
Performance status (ECOG)[a] at entry	
0	8
1	11
2	6
Tumor types	
Colorectal	5
Melanoma	6
Renal cell	5
Mycosis fungoides	1
Other solid tumors	8
Prior therapy	
Irradiation	8
Chemotherapy	15
Hormonal	3
Immunotherapy[b]	11

[a] ECOG, Eastern Cooperative Oncology Group.
[b] Prior immunotherapies include IL-2-based therapy (seven), α interferon (one), γ interferon (one), 5-fluorouracil (one), and monoclonal antibody (one).

following eligibility criteria: histologically confirmed metastatic solid tumor; Karnofsky performance status of ≥70; untreated life expectancy of ≥3 months; ineligibility for surgery, standard chemotherapy, or radiotherapy; WBC count of >3000/mm^3; granulocyte count of >1500/mm^3; platelet count of >100,000/mm^3; serum creatinine level of <1.5 mg/dl; bilirubin level of <1.5 mg/dl; and albumin level of >30 gm/dl.

Patients were excluded for the following reasons: history of coronary artery or clinically significant vascular disease; adult asthma; brain metastases; presence of hepatitis B surface antigen or antibodies to human immunodeficiency virus; infections requiring parenteral antibiotics; pregnancy; chemotherapy, radiotherapy, or hormonal therapy within 4 weeks of entry into the study; or replacement of more than one third of the liver by tumor.

Pretreatment evaluation included the following: history and physical examination; complete blood count; serum chemistry profile; prothrombin time; partial thromboplastin time; fibrin degradation products; urinalysis; chest X-ray and other radiological studies as required to evaluate the extent of tumor; electrocardiogram and cardiac stress test; and pulmonary function tests and arterial blood gases. This protocol was approved by the Institutional Review Boards of both the Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, and the Frederick Cancer Research and Development Center. All patients gave written informed consent.

Drug Supply and Preparation. Polyinosine, polycytosine, poly-L-lysine, and carboxymethylcellulose (poly-ICLC) were formulated by the University of Iowa (15). The final solution contained 2 mg/ml high molecular weight polyinosine/polycytosine, 1.5 mg/ml low molecular weight poly-L-lysine, and 0.5% carboxymethylcellulose. The drug was diluted in endotoxin-free isotonic saline for i.m. injection. IL-2 was provided by Hoffmann-LaRoche (Nutley, NJ) and measured in international units.

Study Design. This study was designed to examine the immunomodulatory properties of poly-ICLC at the lowest dose (1.0 mg/m^2) found to have immunological effects in a previous study (5) and at four other lower dose levels, *i.e.*, 1.0, 0.3, 0.1, 0.03, and 0.01 mg/m^2. Poly-ICLC was given by i.m. injection alone and in combination with IL-2. The immunomodulatory effects of poly-ICLC alone were assessed for the first 4 weeks. A single dose of poly-ICLC was given, followed by a 1-week rest. Patients then received poly-ICLC injections two times per week in weeks 2–4. Then, for 8 additional weeks, patients were treated i.m. with poly-ICLC at the same dose, followed immediately by IL-2 (3×10^6 units/m^2, by 24-h continuous i.v. infusion) twice weekly. All patients were treated in the outpatient setting. IL-2 was infused via a central venous catheter, usually attached to a s.c. venous access device (Port-A-Cath, Infusaid, Inc., Norwood, MA) using an ambulatory portable infusion pump (Shiley Infusaid, Inc., Norwood, MA). These 3 months constituted one treatment cycle. Patients were considered evaluable for response if they completed one cycle (3 months) of therapy. Tumor measurements were recorded at monthly intervals, and standard response criteria were used. Patients were removed from the study if they developed progressive disease after 3 months of therapy or if progressive disease requiring palliative therapy occurred during the initial 3 months of therapy.

All patients received acetaminophen, 650 mg p.o., every 4 h and indomethacin, 25–50 mg p.o. or per rectum, every 8 h during IL-2 infusions, to reduce fever. Meperidine, diphenhydramine, and antiemetics were used as needed, to treat rigors, pruritus, and nausea and vomiting, respectively.

Toxicity Assessment. Toxicities were graded according to the Biological Response Modifiers Program-modified NCI Cancer Therapy Evaluation Program common toxicity criteria. Toxicities were graded and recorded daily for the first 4 days of treatment with poly-ICLC alone. Thereafter, patients were evaluated twice weekly in weeks 2 and 3 (poly-ICLC) and weeks 5 and 8 (poly-ICLC plus IL-2). Physical examinations were performed before and 48 h after the initial dose of poly-ICLC and the initial IL-2 infusion. A complete blood count, chemistry panel, and urinalysis were performed in weeks 1 and 3 (poly-ICLC) and weeks 5 and 8 (poly-ICLC plus IL-2).

Immunological Monitoring. Immune parameter testing was performed on serum and PBM obtained from patients during the week before treatment (two or three samples) and at 0, 24, 48, and/or 72 h following the first poly-ICLC injection in weeks 1, 3, 5, 8, and 10. Whole blood was collected in preservative-free heparin, and Ficoll-Hypaque-isolated PBM were used fresh (for cytotoxicity assays) or were cryopreserved, in RPMI 1640 (GIBCO) supplemented with 10% fetal calf serum (Whittaker Bioproducts, Walkersville, MD) and 7.5% dimethyl sulfoxide (Fisher, Silver Spring, MD), using a Cryo-Med controlled-rate freezer (Mt. Clemens, MI), and stored in liquid nitrogen. Serum was separated and frozen at −70°C.

Cytotoxicity Assays. NK cell assays were performed as standard 4-h ^{51}Cr release cytotoxicity assays (16), using the human chronic myelogenous leukemia K562 cell line as target. Specific cytotoxicity was calculated as the percentage of ^{51}Cr released in the experimental group minus the percentage released in the medium control. Data are expressed as lytic units, calculated from the specific cytotoxicity at effector to target ratios of 50:1, 25:1, 12.5:1, and 6:1 (17). One lytic unit is defined as the number of effector cells required to lyse 1000 target cells, and the data are expressed as lytic units/10^7 effector cells.

Flow Cytometry. Phenotyping of all samples from an individual patient was performed on a single day, to minimize variability in staining and instrument calibration. Frozen mononuclear cells were thawed and resuspended in PBS containing 2% heat-inactivated pooled human AB serum, for 5 min at room temperature, to block Fc receptors or in PBS containing 2% bovine serum albumin for anti-CD16 staining. Fluorescein- or phycoerythrin-conjugated antibodies to CD16, CD3, CD4, CD45, CD57, CD56, CD25, HLA-DR, CD8, CD14, and CD38, as well as unconjugated anti-CD56, were obtained from Becton-Dickinson Immunocytometry Systems (Mountain View, CA). Anti-CD11b was obtained from Coulter Corporation (Hialeah, FL). Cells incubated with unconjugated primary antibodies were stained with fluorescein-conjugated goat anti-mouse immunoglobulin (Tago Inc., Burlingame, CA). Residual RBC were lysed by brief incubation in buffered ammonium chloride, and the samples were fixed with buffered 1% paraformaldehyde in PBS.

Flow cytometric analysis was performed on a Coulter Profile flow cytometer, with four-decade logarithmic amplification (Coulter Cytometry, Hialeah, FL). Lymphocyte and monocyte populations were bitmap gated based on light scatter analysis. Gating effectiveness was confirmed by two-color analysis of CD45-fluorescein- plus CD14-phycoerythrin-stained cells. The percentage of positive cells staining for a given antibody or antibody combination was determined by comparison to isotype controls.

2–5A. 2–5A activity was measured as described by Merritt *et al.* (18). PBM were lysed and incubated with polyinosine/polycytosine-agarose beads, to allow adherence of the 2–5A enzyme. Beads were then

incubated for 20 h with [^{3}H]-ATP buffer solution, followed by digestion with bacterial alkaline phosphatase. Five-μl aliquots were spotted onto DEAE-cellulose paper disks, washed, eluted with 0.3 M KCl, and counted. Specific activity is defined as pmol of ATP incorporated per 10^5 cells per h. One unit of enzyme activity is defined as the incorporation of 1 pmol of ATP per h at 37°C.

Neopterin. Serum levels of neopterin were measured using a radioimmunoassay kit (Neopterin-RIAcid; HENNING Berlin GmBH), according to the manufacturer's instructions. The upper limit of the normal range is approximately 10 nmol/liter.

Interferon. γ Interferon was assayed by a sandwich radioimmunoassay kit (Gamma Interferon Radioimmunoassay; Centocor, Inc., Malvern, PA), according to the manufacturer's instructions. Normal serum does not contain detectable levels of γ interferon. α Interferon serum levels were determined by inhibition of the cytopathic effect of vesicular stomatitis virus on the bovine kidney MDBK cell line (American Type Culture Collection, Rockville, MD), as described previously (19).

IL-2R. Serum IL-2R levels were determined using a commercially available enzyme immunoassay (CELLFREE Interleukin-2 Receptor Test Kit; T Cell Sciences, Cambridge, MA). The upper limit of IL-2R values for normal individuals, as reported by the manufacturer, is 477 units/ml.

Statistical Analysis. Data in this study were evaluated using standard analysis of variance and repeated measures analysis of variance. For some analyses (hematological, cytotoxicity, neopterin, and 2-5A assays), data were transformed to their common logarithms, to satisfy homogeneity of variance and covariance requirements. *Post hoc* comparisons were carried out using Bonferroni control for type I error rates (20).

RESULTS

Patient Population. The characteristics of the 25 patients treated with poly-ICLC plus IL-2 are outlined in Table 1. Three patients received at least two full cycles of treatment, and 14 additional patients completed one cycle of treatment. Eight patients received less than one treatment cycle because of progressive disease (six patients), grade 3 fatigue (one patient), or patient refusal (one patient). Four of five patients developed progressive disease during treatment with poly-ICLC alone at the 0.03 mg/m^2 dose and never received combined treatment with poly-ICLC and IL-2.

Clinical Antitumor Activity. No partial or complete responses were observed. One patient with renal cell cancer had a minor response by the 12th week of treatment with IL-2 and poly-ICLC at the 1.0 mg/m^2 dose level, and he was treated until he developed progressive disease at 26 weeks. Two patients (one each with colon cancer and rectal cancer) had stable disease and received an additional 3-month cycle of therapy but then developed progressive disease.

Clinical Toxicity. Treatment with poly-ICLC alone and in combination with IL-2 was well tolerated at all doses. The most prominent toxicities associated with poly-ICLC alone were lymphopenia (grades 2–4), fatigue, fever, hypotension, nausea/vomiting, and pain at the injection site (all grade 1 or 2). These were present at all poly-ICLC doses and had either resolved or begun to return to normal levels within 24 h. Although toxicity increased with the addition of IL-2, therapy remained tolerable. During combined therapy, patients at all doses experienced transient lymphopenia (grades 3 or 4), fever, fatigue, skin erythema, chills, peripheral edema, creatinine elevations, and diarrhea. Some patients also had arthralgia or myalgia. Clinically significant hypotension was not prominent at this low dose of IL-2. All toxicity was grade 1 or 2, except for lymphopenia, fever (grade 4 in one patient, grade 3 in two), fatigue (grade 3 in two), and vomiting (grade 3 in one). All toxicities were resolving within 1 day of discontinuation of IL-2 and did not appear to be related to the poly-ICLC dose. The toxicities of combined poly-ICLC and IL-2 treatment were consistent with our experience with IL-2 alone at this dose and schedule.

Hematological Effects. Following treatment with poly-ICLC alone or in combination with IL-2, lymphocyte counts were significantly decreased at 24 h; however, they increased over time and were found to be increased significantly from base line on day 49 ($P = 0.0018$). No other significant dose-related changes in total WBC or monocyte counts were noted after treatment with poly-ICLC alone.

Immunological Effects. Four of five patients treated with 0.03 mg/m^2 poly-ICLC developed progressive disease and were removed from study prior to receiving IL-2. Therefore, the immunological effects of combined therapy with poly-ICLC plus IL-2 at the 0.03 mg/m^2 dose of poly-ICLC cannot be determined.

NK Activity. NK activity was measured before and 24 and 48 h after the first and fourth dose of poly-ICLC alone and the first, seventh, and 11th dose of poly-ICLC given in combination with IL-2. Combined therapy was initiated on day 28. Peripheral blood NK activity was not increased after i.m. injection of poly-ICLC alone, regardless of the dose (data not shown). However, it was enhanced during combined treatment. Fig. 1 depicts the level of NK activity in the peripheral blood after 20 days of treatment with both poly-ICLC and IL-2 (day 49). Due to small patient numbers and the loss of all but one patient at the 0.03 mg/m^2 level, patient data were combined into two groups, those receiving 0.3 or 1.0 mg/m^2 poly-ICLC (described as $\geq$0.3) and those receiving 0.01, 0.03, or 0.1 mg/m^2 poly-ICLC (described as $\leq$0.1). Analysis showed no significant increase in NK activity from base line on day 49 in the $\leq$0.1 combined dose group ($P = 0.1079$); however, there was a significant increase from base line on day 49 in the $\geq$0.3 combined dose group ($P = 0.0006$). The difference in NK activity on day 49 between the $\leq$0.1 and the $\geq$0.3 dose groups was also significant ($P = 0.0152$).

Flow Cytometry. No consistent changes in the phenotype of peripheral blood mononuclear cells were observed during treatment with poly-ICLC alone, at any dose or time point tested. However, repeated measures analysis demonstrated a statistically significant increase in CD56$^+$ cells following IL-2 treatment ($P = 0.0005$) (Fig. 2). In addition, there was an increase in CD56 staining intensity, resulting in an increase in the percentage and number of cells having a CD56^{bright} phenotype. This increase in CD56$^+$ cells was associated with changes in the distribution of CD56 subsets recognized by two color combinations of CD56/CD16 and CD56/CD8. As shown in Fig. 3*A*, at baseline the majority of CD56$^+$ cells have weak staining intensity with CD56 and coexpress CD16. Following treatment with poly-ICLC and IL-2, CD56$^+$ cells can be divided into two subsets, based on CD56 staining intensity (Fig. 3*B*); the CD56^{dim} subset is primarily CD16$^+$, while the CD56^{bright} subset is predominantly CD16$^-$ but includes both CD8$^+$ and CD8$^-$ cells (Fig. 3*D*). A small fraction of these cells may also have weak expression of CD16 (Fig. 3*B*). The majority of patients had changes in CD56 phenotype comparable to those depicted in Fig. 3, *B* and *D*, a decrease in the percentage of CD56$^+$ cells coexpressing CD16 and an increase in the CD56$^+$/CD8$^+$ subsets of cells. Changes in CD56 staining intensity and subset distribution occurred during the poly-ICLC plus IL-2 phase of treatment and were independent of the poly-ICLC dose. Corresponding increases in the percentage of CD11b$^+$ and CD38$^+$

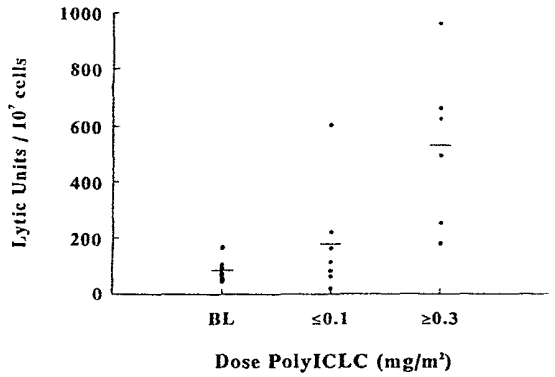

Fig. 1. NK activity on day 49 (21 days after initiating combined therapy) of therapy for patients treated with ≥0.3 or ≤0.1 mg/m² poly-ICLC plus 3 × 10⁶ units/m² IL-2. *Circles*, lytic units measured for each individual patient. *Horizontal lines*, mean lytic activity for each dose group ± SE. *BL*, baseline cytotoxicity level for all patients.

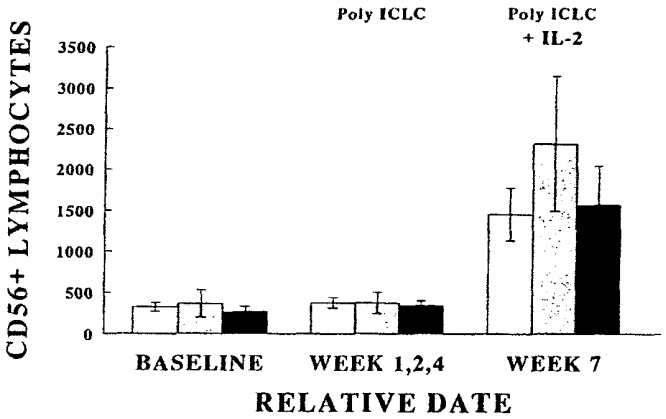

Fig. 2. Changes in the number of CD56⁺ peripheral blood lymphocytes during therapy with poly-ICLC alone (weeks 1, 2, and 4) and in combination with IL-2 (week 7). Data are presented as absolute number of CD56⁺ cells ± SE. Baseline values were from five patients at each dose level. During poly-ICLC alone, there were five patients at 0.3 mg/m² and four patients at 1.0 mg/m². During combined therapy, data were available for five patients at 0.1 mg/m², two patients at 0.3 mg/m², and four patients at 1.0 mg/m². Treatment with poly-ICLC plus IL-2 was associated with significant increases in CD56⁺ cells independently of the dose of poly-ICLC. □, 0.1 mg/m²; □, 0.3 mg/m²; ■, 1.0 mg/m².

cells and an increase in staining intensity for CD38 were also observed (data not shown). The percentage of CD3⁺ cells decreased in proportion to the increase in CD56⁺ cells; however, the absolute number of CD3⁺ cells/mm³ increased or showed no change. The percentage of CD4⁺ cells declined in parallel with CD3, while the percentage of CD8⁺ lymphocytes remained unchanged or increased, due to the increased percentage of CD8⁺/CD56⁺ cells. When the percentage of CD8⁺/CD56⁻ T cells was determined, no change in the ratio of CD4⁺ to CD8⁺ T cells was detected. All of these changes were independent of the dose of poly-ICLC.

2',5'-Oligoadenylate Synthetase. As shown in Fig. 4*A*, 72 h after the initial poly-ICLC dose, the mean percentage of baseline 2–5A activity of PBM obtained from patients receiving 1.0 mg/m² poly-ICLC was significantly elevated above the mean percentage of baseline activity of cells from patients receiving lower doses of poly-ICLC. Pairwise comparisons between the 1.0 mg/m² dose group and the remaining dose groups yielded *P* values of 0.0224, 0.0006, and 0.004, respectively, while comparison between the 1.0 mg/m² dose group and the other three groups taken collectively was also significant ($P = 0.0010$). While small elevations were observed in individual patients treated with 0.3, 0.1, and 0.03 mg/m² poly-ICLC, the mean percentage of baseline 2–5A activity levels for these dose groups were not significantly different from one another or from base line. Although patients treated at 1.0 mg/m² had significant elevations over base line 72 h after poly-ICLC therapy, the actual values of 2–5A activity observed in these patients were, with one exception, within the range measured for patients treated at lower doses. Because of patient drop-out and a small number of samples available at comparable time points (*i.e.*, 72 h after poly-ICLC administration), there are insufficient data to compare the effects of combined *versus* poly-ICLC only therapy on 2–5A synthetase activity.

Neopterin. As shown in Fig. 4*B*, serum neopterin levels in patients 72 h after treatment with 1.0 mg/m² poly-ICLC alone were significantly elevated, compared to patients treated at lower poly-ICLC doses ($P < 0.05$ *versus* individual dose groups, $P = 0.0012$ *versus* combined). There were no significant differences in neopterin levels among patients at the three lower dose levels. Following initiation of IL-2 therapy, circulating neopterin values for all patients, regardless of poly-ICLC dose, were elevated over base line and rose higher than neopterin levels obtained by treatment with poly-ICLC alone. No significant differences were observed between any of the poly-ICLC dose groups during combined therapy (data not shown).

Interferon. Despite the proven ability of poly-ICLC to induce type I interferons (13), neither α nor γ interferon was detected in serum samples drawn 24, 48, or 72 h following treatment with poly-ICLC alone or in combination with IL-2 (data not shown). This was true regardless of the poly-ICLC dose.

Interleukin 2 Receptor. Soluble IL-2R levels remained unchanged during treatment with poly-ICLC alone, regardless of the dose, but were increased following initiation of IL-2 treatment (data not shown). The soluble IL-2R levels achieved during combination therapy were similar in all groups and appeared to be related solely to IL-2 administration.

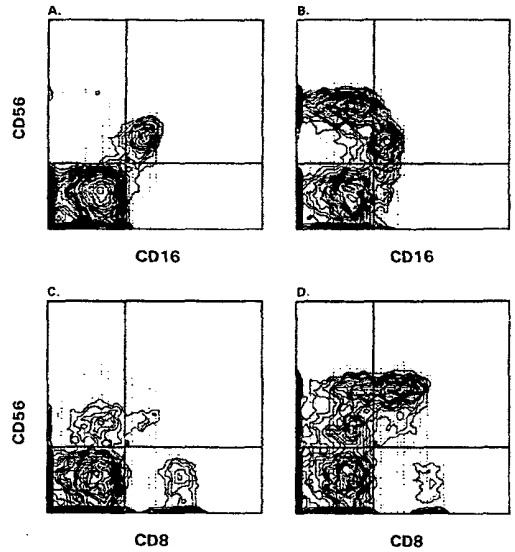

Fig. 3. Changes in CD56⁺ subsets associated with the IL-2 phase of therapy. *A* and *C*, baseline phenotype, in which the majority of CD56⁺ cells have weak staining intensity for CD56 (CD56^{dim}) and coexpress CD16. *B* and *D*, sample obtained from the same patient on day 49 of therapy with 1.0 mg/m² poly-ICLC plus IL-2. There is an increase in the CD56^{bright} subset; these cells include both CD8⁺ and CD8⁻ subsets, and some also stain weakly for CD16.

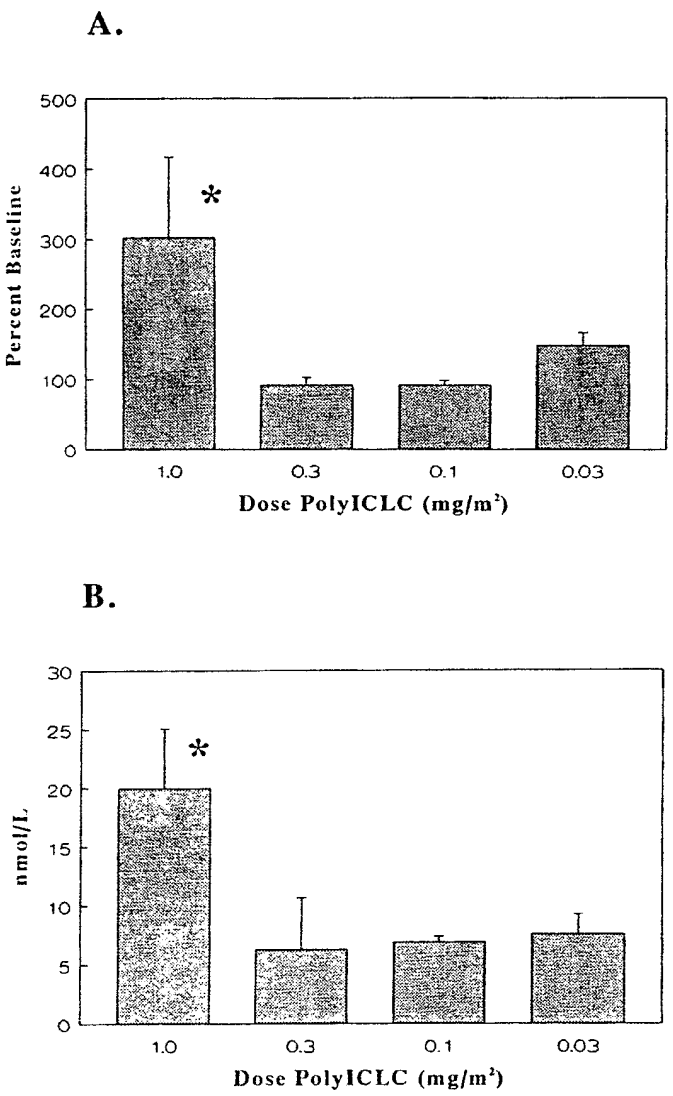

Fig. 4. *A*, mean levels of 2–5A generation 72 h following initial poly-ICLC injection. Data are expressed as percentage of baseline levels of 2–5A measured. *Error bars*, ±SE. Data are from three, two, four, and four patients at the 1.0, 0.3, 0.1, and 0.03 mg/m² dose levels, respectively. *, $P = 0.0001$. *B*, mean levels of serum neopterin (nmol/liter) measured 72 h after initial poly-ICLC injection. *Error bars*, SE. Data are from five, two, four, and five patients at the 1.0, 0.3, 0.1, and 0.03 mg/m² dose levels, respectively. *, $P = 0.0012$.

DISCUSSION

Experimental animal studies have shown that poly-ICLC treatment at doses below the MTD results in greater immunomodulation and superior antitumor effects (2). Clinical trials of poly-ICLC at the MTD have documented minor antitumor activity (3), but minimal data are available regarding efficacy at lower, and perhaps more immunologically effective, doses of poly-ICLC. Because a previous trial of poly-ICLC found the greatest immunomodulatory activity at the lowest dose tested (1 mg/m²), we administered doses of poly-ICLC of ≤1.0 mg/m², to see whether lower doses would have greater immunological and therapeutic effects.

Treatment with poly-ICLC alone was well tolerated at all doses levels, but few immunological effects were documented. The majority of immune parameters exhibited changes only after the initiation of IL-2 treatment. Only serum neopterin levels and 2–5A synthetase activity were elevated with poly-ICLC alone, and those only at the 1 mg/m² dose level. This dose has previously been shown to induce type I interferons (15), but we could not detect circulating α interferon at any dose. One possible explanation is timing; since only 24-h time points were drawn, it is possible that serum interferon levels peaked early and returned to base line by 24 h. In any case, elevated 2–5A activity and serum neopterin levels, such as those demonstrated in this study, have been reported to be sensitive indicators of the presence of low levels of interferon (21). Neither of these parameters exhibited significant changes at any time with any of the lower poly-ICLC doses. Therefore, according to the immune function tests performed in this study, 1 mg/m² appears to be the optimal immunological dose of the poly-ICLC doses tested.

A second objective was to determine whether poly-ICLC could augment the immunostimulatory effects of IL-2. In contrast to treatment with poly-ICLC alone, within 3 weeks of the addition of low dose IL-2 to the treatment regimen we were able to demonstrate an elevation in the percentage and absolute number of circulating $CD56^+$ cells in all patients, as well as changes in their subset distribution . Additionally, soluble IL-2 receptor and neopterin levels were significantly elevated over base line. No difference in any of these parameters was detected between the poly-ICLC dose groups. All of these findings have been reported to occur following IL-2 treatment alone (22, 23). Our findings indicate that the coadministration of poly-ICLC with IL-2 does not alter the spectrum of immune changes observed, but it enhances the NK activity observed after treatment with low to moderate doses of IL-2 alone.

Low dose IL-2 can enhance NK and LAK activity, but it requires a longer period of administration to achieve immunomodulation comparable to that of high dose therapy. IL-2 at doses as low as 1×10^6 units/m², given by daily i.m. injection, has been shown by us to result eventually in markedly enhanced NK activity (22). Our experience employing high and low dose regimens of IL-2 indicates that the overall number of effector cells and, therefore, total lytic activity are higher after treatment with high dose IL-2. Thus, one advantage of high dose IL-2 regimens appears to be the ability to generate a larger pool of potential effector cells. Our data suggest that, by combining IL-r2 treatment with poly-ICLC at doses of ≥0.3 mg/m², a population of cells is generated that is significantly more lytic than cells generated at lower doses of poly-ICLC plus IL-2. This augmentation of IL-2 effects may be of therapeutic value. High dose IL-2 (*i.e.*, 30×10^6 units/m²) is an effective immunostimulatory agent that results in increased numbers of $CD56^+$ cells and NK/LAK activity (7), but treatment is associated with severe toxicity requiring hospitalization and significant supportive care. Lower doses of IL-2 ($\leq 3 \times 10^6$ units/m²) are normally well tolerated, may be given as an outpatient regimen, and, as seen in this study, can be combined with poly-ICLC with minimal changes in the spectrum or severity of IL-2 toxicities.

These data suggest that it may be possible to increase the lytic activity of circulating peripheral blood mononuclear cells by adding 1.0 mg/m² poly-ICLC to a low dose IL-2 regimen. While increased lytic activity, as signalled by elevations in NK or LAK cells, has yet to be shown to translate into antitumor activity, our observations raise the possibility that additional combined therapies utilizing the combination of IL-2 and poly-ICLC with, for example, a monoclonal antibody or a chemotherapeutic agent may be of clinical benefit. It is also conceivable that the optimal combination observed in this study (poly-

ICLC at 1 mg/m^2 plus IL-2 at 3×10^6 units/m^2) might be active in a phase II study of earlier stage patients.

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